Swami Ramanand Teerth Marathwada University, Nanded

A Synopsis on

The Proposed Research Work Leading to the Degree of

Doctor of Philosophy In

Microbiology

Entitled

"Studies on Plant Growth Promoting Potential of Mangrove Soil Bacteria and its Application for Increasing Yield of Wheat *(Triticum aestivum, L)*"

Submitted by

Gaikwad Kulbhushan Rangnath

M. Sc. Microbiology

CSIR-UGC NET, Maharashtra SET

Under the guidance of

Dr. D.V.Vedpathak

M. Sc., Ph. D.

Department of Microbiology

Rajarshi Shahu Mahavidyalaya (Autonomous)

Latur – 413 512

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Introduction:

Due to the negative consequences of climate change, global warming, and increased environmental pressures, agricultural output is currently dropping. As a result, in order to achieve sustainable agricultural development and increase agricultural products to feed the entire world's population, it appears that appropriate solutions and ecologically compatible and environmentally friendly techniques must be used to reduce the negative effects of these stresses on plants.

Soil salinization is a process of localised accumulation of soluble salts. This phenomenon is now widely regarded as a serious threat to agricultural fields, as it directly degrades soil value and quality. Salts containing anions like chloride, sulphate, and nitrate, as well as sodium and potassium cations, promote soil salinization. Plants are harmed by salinity because it interferes with photosynthesis, respiration, assimilate distribution, and causes wilting, drying, and death of entire organs. High salinity makes plants more susceptible to a variety of phytopathogens and encourages the spread of several fungal soil-borne illnesses. PGPRs, which can manufacture the aforementioned lytic enzymes, siderophores, IAA, and antibiotics, provide an alternative to using agrochemicals. Phytopathogens that cause plant infections could be reduced and inhibited by these substances. (Anna Otlewska, 2020)

Around the world, soil salinity is an issue for agricultural productivity. Crops that grow in salt-affected soils are prone to osmotic stress, poor physical soil conditions, nutritional disorders and toxicity, and reduced crop yields, according to current reports. Around the globe, 20% of irrigated land is severely damaged by salt accumulation (Selvakumar et al., 2014). This land deterioration is expected to reach up to 50% by the year 2050 (Hossain, 2019).

To deal with the backdrop of rising food demands, limiting crop losses due to salt stress is one of the serious challenges before agricultural practices. Crop yield is principally depending upon provision of nutrients to the crop and its protection from diseases. To boost food production on salt-affected soils, new agricultural technologies are required. Beneficial halotolerant rhizospheric bacteria have been shown to improve plant salinity tolerance through mechanisms such as root system development, improved soil structure, increased water and nutrient uptake, reduced sodium absorption and reduced negative effects of stress ethylene.

Wheat (Triticum aestivum L.) is a staple food for the human population of majority part of the world. However, there is an immense need to enhance crop productivity to meet ever increasing food demands by the year 2050 in parallel with continuous shrinking of cultivable land holding and land loss caused due to increasing salinity per year.

To ensure food security for the people, the Indian government has set a goal of repairing 26 million hectares of degraded farmland, including salt-affected soils, by 2030. The country's salt-affected land is estimated to be 6.74 million hectares. According to estimates, approximately 10% more land is becoming salinized each year, and by 2050, nearly half of all arable land will be contaminated by salt. Saline soils cover 44% of the land in 12 states and one union territory, whereas sodic soils cover 47% of the land in 11 states.

Microbial inoculation to relieve plant stress could be a more cost-effective, environmentally friendly solution that could be provided in a shorter amount of time. Such inoculants aid in the growth of long-term agriculture in salinity-stressed environments. Exploitation of root adherent plant growth promoting rhizobacteria (PGPR) inhabiting hyper-saline circumstances has recently gained traction as an alternative eco-friendly biological strategy to improve crop output on salt-degrading lands. (Talaat, 2015). Plant growth promotion and yield increase aided by these microbes is well documented (Barnawal et al., 2012; Bharti et al., 2014).

The plant growth promoting potential through provision of nutrients to plants and biocontrol of phytopathogens of rhizospheric bacteria from saline soils is needed to be more explored. Salt tolerant -PGPR and their potential in enhancing the productivity of saline agro-ecosystems by PGPR mediated mechanisms of salt tolerance in different crop plants and future research trends of using ST-PGPR for reclamation of saline soils are also highlighted. (Dilfuza, et.al., 2019)

This study will be helpful to explore the potential of salt tolerant strains of PGPR from saline soil environments and further their utilization as biofertilizer for wheat crop to minimize yield losses due to salt and fungal phytopathogens induced stress.

The Mangrove Ecosystem:

The mangrove ecosystem is a salty woodland or shrubland habitat with a coastal environment that collects fine sediments in regions protected from high wave activity. The ecosystem aids in the preservation of coastal fisheries, but in many nations, systematic

devastation is on the rise. Microbial community restoration is essential for the protection of the mangrove ecosystem. Microbe-nutrient-plant connections are researched for recycling and preserving vital nutrients in the mangrove ecosystem, which is the best studied process. The mangrove ecosystem is rich in organic matter, but it is widely thought to be nutrient poor, especially in nitrogen and phosphorus, which are critical nutrients for plant growth.

In tropical mangroves, bacteria and fungi account for 91% of total microbial biomass, while algae and protozoa account for just 7% and 2%, respectively. Bacteria colonizing plant root surfaces cause root exudation, which drives microbial activity by providing bacteria with a source of food whereas root exudates in mangroves fuel microbial communities in sediments (Alongi et al 1994). Mangrove trees can also change the soil's properties and oxidise it by supplying oxygen via their aerial roots (Sherman et al 1998).

Nitrogen fixers, phosphate solubilizers, siderophore makers, phytohormone producers, and other plant growth promoting bacteria (PGPB) are among the bacteria examined in conjunction with crops. The bacteria are from different genera and can be employed separately or in combination to inoculate plants and boost their growth. Pneumatophores, which fix nitrogen, are particularly widespread in the mangrove ecosystem (aerial roots). *Azospirillum, Azotobacter, Rhizobium, Clostridium,* and *Klebsiella* are nitrogen-fixing species found in this habitat (Sengupta and Choudhari, 1990).

Due to the abundance of cations in the marine environment, phosphorous precipitates and becomes inaccessible, however some strains isolated from the mangrove habitat are capable of phosphorous solubilization (Vazquez et al, 2000). A phosphate-solubilizing strain from such a mangrove habitat is *B.amyloliquifecians*. The usage of PGPB to boost agricultural output is a standard practise. Mangrove roots have PGPB potential (Holguin, et al 2001) and could be used to promote plant development. In addition, research have demonstrated that mixed cultures outperform pure cultures in terms of plant growth promotion. In the mangrove ecosystem, a mixed culture of nitrogen-fixing bacteria *Phylobacterium sp.* And PSB *Bacillus licheniformis* tripled nitrogen fixation compared to a pure culture.

The present dissertation hypothesizes to evaluate the plant growth promoting potential of mangrove soil bacterial isolates to increase yield of wheat (*Triticum aestivum L*) growing in saline soils.

Review of Literature:

National Relevance of the Study:

Pseudomonas, Bacillus, Enterobacter, Agrobacterium, Streptomyces, Klebsiella, and *Ochromobacter* are the most commonly reported bacterial genera for increasing agricultural yield under saline circumstances. (Sharma et al., 2016; Singh and Jha, 2016). Diazotrophic salt-tolerant bacterial strains of *Klebsiella, Agrobacterium, Pseudomonas*, and *Ochrobactrum* isolated from the roots of a halophytic plant, *Arthrocnemum indicum*, enhanced peanut productivity in both saline and control environments. (Sharma et al., 2016). Under salinity stress, *Planococcus rifietoensis*, an alkaliphilic bacterium, has been shown to improve wheat crop development and productivity. (Rajput et al., 2013). Upadhyay et al. (2009) explored the genetic diversity of ST-PGPR isolated from the wheat rhizosphere. They discovered that the majority of the isolates could tolerate up to 8% NaCl and belonged to the Bacillus genus. In the soil of India's eastern Indo Gangetic plains, the diversity of salt-tolerant bacilli was also discovered by Sharma et al. (2015). They isolated 95 bacterial strains and amongst them, 55 showed plant growth promoting characteristics and salttolerance to more than 4% NaCl. Several researchers also report the diversity of ST-PGPR in the coastal areas. For example, 121 bacterial strains were identified in Tsunami-affected areas of India's Andaman and Nicobar Islands, and 23 of them demonstrated salt resistance up to 10% NaCl with PGP features such as synthesis of indole acetic acid (IAA), siderophore, extracellular enzymes, and phosphate solubilization. (Amaresan et al., 2016).

International Relevance of the Study:

Microorganisms belonging to several groups of bacteria, fungus, and archaea can be found in soil. Some microorganisms are now well known for their natural capacity to tolerate various salt concentrations while still promoting plant growth. In agriculture, these salt-tolerant plant beneficial bacteria are extremely important. They've demonstrated their ability to boost agricultural productivity in dry and semiarid environments. (Niu et al., 2018). Studies confirmed that ST-PGPR produce various types of phytohormones, such as auxins, gibberellins, cytokinins (Dodd et al., 2010), synthesize ACC deaminase (Glick et al., 2007), produce secondary compounds such as exopolysaccharides (Timmusk et al., 2014) and osmolytes (proline, trehalose, and glycine betaines) (Bano and Fatima, 2009), regulate plant defense systems and activate plant’s antioxidative enzymes under salt stress (Hashem et al.,

2016) The diversity of salt-tolerant bacteria isolated from paddy rhizosphere in Taoyuan, China was reported by Zhang et al. (2018). They isolated 305 bacterial strains, and amongst them, 162 were tested for salt tolerance up to 150 g/l NaCl concentration. Phylogenic analysis of 74 of these salt-tolerant strains showed that they belong to orders Bacillales (72%), Actinomycetales (22%), Rhizobiales (1%), and Oceanospirillales (4%). Most of the isolates also showed their potential in improving salt tolerance, growth, and yield of rice under salt-stress conditions. ST-PGPR strain *Bacillus licheniformis* SA03 isolated from Chrysanthemum plants grown in saline-alkaline soil of China conferred increased salt tolerance in Chrysanthemum (Zhou et al., 2017). In another study, whole genome analysis of a halotolerant PGPR Klebsiella sp. D5A revealed the presence of salt tolerance genes with a wide range of pH adaptability and PGP traits including phosphate solubilization, IAA biosynthesis, acetoin, and 2,3-butanediol synthesis, siderophore production, and N2 fixation (Liu et al., 2016). *Pseudomonas putida* and *Novosphingobium* sp. are reported to reduce salt-stress induced damage in citrus plants by lowering the level of abscisic acid (ABA) and salicylic acid (SA), reducing the efficiency of photosystem II (Fv/Fm), increasing accumulation of IAA in the leaf and inhibiting accumulation of root chloride and proline during salt stress (Vives-Peris et al., 2018). Under salt stress, a *Pseudomonas* strain isolated from the halophilic grass Distichlis spicata also boosted the development of other crops. (Palacio-Rodríguez et al., 2017). Through induction of reactive oxygen species (ROS) scavenging enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) and upregulation of ROS pathway genes, a ST-PGPR strain Enterobacter sp. UPMR18 with the ability to produce 1 aminocyclopropane -1-carboxylic acid (ACC) deaminase improved crop productivity. (Habib et al., 2016)

Objectives:

1) To collect soil samples from saline soils and mangrove soils of Maharashtra.
2) To isolate bacterial strains from these saline soil samples.
3) To screen the selected bacterial strains against soil born fungal phytopathogens.
4) To Screen the different bacterial isolates for salt tolerance potential.
5) To identify the bacterial isolates by PCR & Sequencing.
6) To study the Plant growth promoting traits of bacterial isolates such as
    - Phosphate Solubilization Potentials
    - Siderophore production and its characterization
    - Production and characterization of Indole-3-Acetic acid (IAA) and other auxins, if any
    - Production of fungal cell wall degrading enzymes and their antifungal activity
7) To study production of abscisic acid (ABA) and salicylic acid (SA)
8) Pot trials for plant growth promoting potential by selected salt tolerant bacterial isolates in Wheat (*Triticum aestivum* L.)
9) Field trials for plant growth promoting potential by selected salt tolerant bacterial isolates in Wheat (*Triticum aestivum* L.)
10) Statistical analysis of crop yield

Methodology to be adopted:

1. Isolation of different types of Bacterial strains from collected saline soil samples using various media
2. Screening of isolated Bacterial strains for salt tolerance by growing at various salt concentrations.
3. Identification & Characterization (Morphological &Molecular) of different Isolates by using standard protocol or techniques.
4. Screening for antagonistic potential of bacterial isolates against phytopathogenic fungi will be observed on Potato Dextrose Agar and Glucose nitrate salt agar media.
5. Estimation of plant growth promoting traits of identified bacterial isolates such as

   - Phosphate solubilization potential using Pikovaskaya agar medium for and broth.

   - Siderophore production by Universal Chemical assay i.e. Chrome Azurol Sulphonate (CAS) assay.

   - The detection of siderophore type will be carried out by UV-Vis spectroscopy, the Csaky test for hydroxamate type and Arnow's test for catecholate-phenolate type of siderophore

   - Chitinase activity using colloidal Chitin amended medium.

     Microscopic observation will be used to detect degradation of pathogenic fungal cell wall.

   - Pot trials and field trials for plant growth promoting potential by bacterial isolates in Wheat (*Triticum aestivum* L.))will be conducted in saline soils using seed treatment as well as foliar application of secondary metabolites

Proposed work Plan/Formulation and Structure of Study:

- **Year wise Plan of work and targets to be achieved**

  The following plan of study has been proposed to accomplish the aims and objectives of the present study.

- **First Year plan of work**
  1. Study the course-work for research awareness and to enhance the self growth with respect to concept and knowledge of the subject.
  2. Extensive Review of literature related to the topic
  3. The collection and characterization of different saline soil samples.
  4. Isolation of efficient bacterial strains.
  5. Screening of bacterial isolates for salt tolerance
  6. Screening for antifungal potential of bacterial isolates
- **Second Year plan of work-**
  1. Screening of bacterial isolates for production of siderophore and IAA
  2. Standardization of media for production of IAA and Siderophore
  3. Study of fungal cell wall degrading enzymes by bacterial isolates
- **Third Year plan of work-**
  1. Pot trials
  2. Field trials
  3. Statistical analysis
  4. Thesis writing

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Dr. D. V. Vedpathak	**Mr. K. R. Gaikwad**
Research guide	Research scholar